A GROWTH INHIBITION MODEL FOR THIOBACILLUS FERROOXIDANS[1]

Kay L. Shuttleworth and Richard F. Unz[2]

Abstract.-- Acidophilic strains of the genus Thiobacillus play a major role in the genesis of acidic mine drainage. The development of these chemolithotrophs has been shown to be adversely affected by the presence of certain organic acids in laboratory cultures. Conversely, acidophilic heterotrophic bacteria, e.g., members of the genus Acidiphilium, are capable of metabolizing a variety of organic acids. A theoretical model was developed which mathematically depicts a possible interaction between these two physiologically distinct types of microorganisms. The model is based on the Monod kinetics model modified to account for the effects of exogenous inhibitors on the chemolithotroph when co-cultured with the heterotroph. Application of the model assumes that the two types of bacteria can coexist in a chemostat receiving two different limiting nutrients, one for each microorganism. For purposes of illustrating the model, (a) growth of the chemolithotroph and the heterotroph are assumed to be limited by ferrous iron and an organic acid, respectively, and (b) the organic acid is inhibitory to the chemolithotroph. In addition, the predictive value of the model is dependent on the assumptions that there is no product inhibition and the level of ferrous iron is not inhibitory to the heterotroph. In this model, the steady state biomass of T. ferrooxidans is a function of the maximum growth rates and substrate half-saturation constants of both members of the population as well as the inhibitor constant of the chemolithotroph. The model indicates that the growth of T. ferrooxidans may be strongly linked to the growth of other organism(s) capable of removing organic inhibitors.

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[2]K. L. Shuttleworth is a Graduate Assistant and R. F. Unz is Professor of Environmental Microbiology, Program in Ecology and Department of Civil Engineering, The Pennsylvania State University, University Park, PA.

INTRODUCTION

The microbial aspects of acidic mine drainage have been the subject of many investigations (Dugan 1975, Harrison 1978, Wichlacz and Unz 1981). One of the key reactions in the formation of acidic mine drainage is mediated by the chemolithotrophic, iron-oxidizing bacterium,

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Thiobacillus ferrooxidans. This organism obtains energy from the oxidation of ferrous iron or reduced sulfur compounds and sustains the oxidation of pyritic sulfur by providing a continuous supply of the oxidant, Fe^{+3} (Singer and Stumm 1970). Furthermore, hydrolysis of the ferric iron yields the mineral acid, H_2SO_4, which is responsible for the low pH of the drainage.

T. ferrooxidans and other acidophilic thiobacilli are of economic importance because of their role in the genesis and continued production of acidic mine drainage; therefore, many researchers have studied the effects of inhibitors on the thiobacilli (Borichewski 1967, Tuttle and Dugan 1976, Tuttle et al. 1977). Compounds which have been found to inhibit the acidophilic thiobacilli include pyruvate, acetate, succinate, glutamate, and fumarate. In addition, there has been much interest in the inhibitory effects of a detergent, sodium lauryl sulfate (Dugan 1987, Dugan and Apel 1983, Onysko et al. 1984). A proposed scheme for the prevention of acidic mine drainage involves treating coal refuse with sodium lauryl sulfate to inhibit the thiobacilli and thus curtail the production of pollutants which characterize acidic mine waters.

The focus of most of the acidic mine drainage literature has been on the acidophilic thiobacilli; however, acidophilic heterotrophs, e.g., members of the genus Acidiphilium, have recently been isolated from acid-mineral environments (Harrison 1981, Wichlacz et al. 1986). Furthermore, nonacidophilic heterotrophic organisms may be found in gelatinous streamers in acidic mine drainage (Johnson et al. 1979).

The organic carbon utilized by heterotrophs may include compounds that are potentially toxic to the acidophilic thiobacilli. It has been shown that acidophilic heterotrophs can use succinate, glutamate, citrate, fumarate, and other similar carbon sources (Wichlacz et al. 1986), some of which have been shown also to inhibit T. thiooxidans, T. ferrooxidans, or both. Thus a potential exists for the two populations to interact in the environment through one common intermediate.

MODEL DEVELOPMENT

A. Background

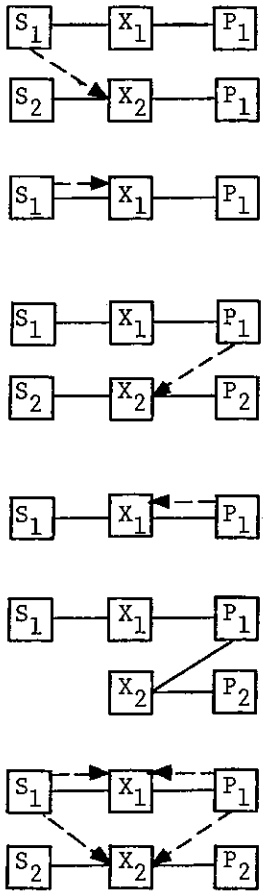
Microorganisms may be inhibited by various compounds, including microbial waste products, high concentrations of substrate, and exogenously supplied chemicals. Ways in which inhibitors can interact with two different species are shown schematically in figure 1. It is possible to develop mathematical models which describe these phenomena, and many investigators have used such models to characterize the effects of product and substrate inhibition on microbial growth (Davison and Stephanopoulos 1986, Luong 1987). Models have been developed which describe how product inhibition can be relieved by the presence of a second organism which removes that product. However, there are apparently few models which describe possible interactions between two species when one of the species is affected by an inhibitor which is not a product of the system.

In this paper, a model will be developed to express the potential interactions between two species of acidophilic bacteria, a chemolithotroph and a heterotroph, cocultured in a continuous culture system. There is no competition for a limiting substrate; however, the chemolithotrophic organism, T. ferrooxidans, is the subject of inhibition by an exogenously supplied organic acid which the heterotrophic organism, an Acidiphilium sp., can degrade. The model may predict potential interactions between the chemolithotroph and the heterotroph in an acidic drainage where exogenous inhibitors are present.

B. Assumptions

Given that T. ferrooxidans is an obligate chemolithotroph and that the Acidiphilium sp. is an acidophilic, obligate heterotroph, it is possible to coculture the organisms in the chemostat by using different growth limiting nutrients for each organism. The specific assumptions are:

(1) The growth limiting nutrient for T. ferrooxidans and for the heterotroph will be ferrous iron and an organic acid respectively.
(2) Growth of T. ferrooxidans will be inhibited by the organic acid.
(3) The iron concentration necessary for the growth of T. ferrooxidans will not be inhibitory to the acidophilic heterotroph (Wichlacz 1980).
(4) There is no product inhibition for either organism.
(5) Given that the mechanism for inhibition of T. ferrooxidans by organic acids appears to be membrane leakage (Tuttle et al 1977), the model will account for an inhibitor which affects both the K_m and u_{max}. This is equivalent to the noncompetitive inhibition model in enzyme kinetics.
(6) The dilution rate for the chemostat is set at a rate which will allow the two species to coexist.



A. Organisms inhibited by substrate of other species.

B. Organisms inhibited by high levels of their own substrate (substrate inhibition).

C. Organisms inhibited by product of other species

D. Organisms inhibited by their own products (product inhibition).

E. Organism 2 uses product of organism 1, i.e., $P_1 = S_2$

F. All possible inhibitory effects.

Figure 1. Schematic of some possible interactions between two species with two substrates and two products. Symbols: substrate 1 (S_1); species 1 (X_1); product 1 (P_1); substrate 2 (S_2); species 2 (X_2); product 2 (P_2); positive effect (————); inhibitory effect (-------).

C. Mathematical symbols

c (subscript)	Chemolithotrophic organism (T. ferrooxidans)
D	Dilution rate (hr^{-1})
h (subscript)	Heterotrophic organism (Acidiphilium sp.)
I	Concentration of inhibitor (moles/l)
K_i	Inhibitor constant corresponding to the dissociation constant of the enzyme-inhibitor complex, moles/l (Michal 1978)
K_m	Half-saturation constant, i.e., substrate concentration when growth rate is equal to ½ maximum rate (moles/l)
L	Loss term, a function of yield (g/g)
M	Biomass concentration (g/l)
S	Substrate concentration in the chemostat (moles/l)
S_o	Substrate concentration in the feed (moles/l)
u	Specific growth rate (hr^{-1})
u_{max}	Maximum specific growth rate (hr^{-1})

D. The Model

The basis for model development will be the Monod model of bacterial growth in a chemostat (Tempest 1970). The Monod model is an equation for saturation kinetics which describes the growth of microbes in terms of a limiting nutrient and factors (K_m and

u_{max}) which are a function of the physiology of the organisms. The chemostat mimics a simplified natural environment with continuous and equal flow rates to and from the system.

In order to interpret data from mixed culture work, it is first necessary to determine the kinetic parameters (K_m and u_{max}) of the organisms in pure culture. Similarily, the K_i for the chemolithotroph must be determined from pure culture studies on T. ferrooxidans in the presence and absence of the exogenous inhibitor. When the continuous culture system is at steady state, there is no net increase in biomass; hence u = D and the relationship of specific growth rate to the other parameters for the system can be expressed for the chemolithotroph and the heterotroph in equations (1) and (2), respectively.

$$(1) \quad u_c = D = \frac{u_{maxc} * S}{K_{ms} + S} - L_c$$

$$(2) \quad u_h = D = \frac{u_{maxh} * I}{K_{mi} + I} - L_h$$

It is necessary to modify equation (1) to include the effects of a noncompetitive inhibitor. In a Lineweaver-Burke linearization of saturation kinetics, it is easy to distinguish the effects of an inhibitor by the apparent K_m and u_{max}. When these inhibitor effects are accounted for and the equation is rearranged into standard form, the result is equation (3).

$$(3) \quad u = \frac{u_{max} * S}{(K_m + S)(1 + I/K_i)}$$

This equation is easily derived and has been credited to Haldane who apparently first described it in 1930 (Luong 1987) and to Aiba and Shoda (Davison and Stephanopoulos 1986). The accuracy of the completed model will be highly dependent on the ability of equation (3) to predict the effects of the inhibitor. It should be noted that Luong (1987) believes equation (3) fails to adequately predict inhibitor effects when the inhibitor and the substrate are the same compound. However, this equation served as the basis for the model of Davison and Stephanopoulos (1986) for product inhibition. Furthermore, the inhibitor of T. ferrooxidans is not an available substrate for the organism.

Equation (3) must be corrected to include loss of cellular material due to cell maintenance. Again steady state will be assumed; therefore, the behavior of an axenic culture of the inhibited chemolithotroph may be described by equation (4)

$$(4) \quad u_c = D = \frac{u_{maxc} * S}{(K_{ms} + S)(1 + I/K_i)} - L_c$$

Thus in axenic culture, growth of the chemolithotroph will be affected by the concentration of inhibitor and the inhibitor constant. In mixed culture, the concentration of inhibitor will be determined in turn by the K_{mi} and the u_{maxh} of the heterotroph as well as the dilution rate.

The limiting substrate for the heterotroph is I and the level of limiting substrate at steady state can be determined by setting equation (5) equal to zero and solving for I. The results of this transformation are given in equation (6).

$$(5) \quad \frac{dM}{dt} = \frac{u_{maxh} * I * M}{K_{mi} + I} - L_h M - DM_h$$

$$(6) \quad I = \frac{K_{mi}(D + L_h)}{u_{maxh} - (D + L_h)}$$

Substituting equation (6) into (4) yields equation (7) which shows the relationship between the chemolithotroph and the heterotroph.

As stated above, the model given in equation (7) includes the simplifying assumption that (I) is not inhibitory to the heterotroph at any concentration. However, acidophilic heterotrophs can be inhibited by high levels of organics (Harrison 1981). Luong (1987) has suggested that equation (8) represents a good model for substrate inhibition. S_m is the maximum substrate level which does not cause inhibition and n is determined empirically. In order to use this model, one would have to solve equation (8) for S and substitute this back into equation (4) as given above. However, solving equation (8) for S by setting dM/dt = 0 is complicated and beyond the scope of this paper. Hence, the model developed here is appropriate only when $I \leq S_m$. This appears to be reasonable since the levels of organics in acidic mine drainage are typically very low (0.003%; Johnson et al. 1979) and the high levels (>0.5%) of organics referred to by Harrison (1981) are typical for culture media used in conventional microbiology.

It is of interest to learn how the inhibitor affects the steady state population of the chemolithotroph. This requires that equation (9) be set equal to

$$(7)\quad u_c = \left(\frac{u_{maxc} * S}{(K_{ms} + S)\left[1 + \frac{K_{mi}\,(D + L_h)}{K_i \left\langle u_{maxh} - (D + L_h) \right\rangle}\right]} \right) - L_c - D$$

$$(8)\quad \frac{dM}{M * dt} = \frac{u_{max} * S}{K_m + S}\,(1 - S/S_m)^n$$

$$(9)\quad \frac{dM_c}{dt} = \frac{u_{maxc} * M_c * S}{(K_{ms} + S)\,(1 + I/K_i)} - (D + L_c)M_c$$

$$(10)\quad S = \frac{K_{ms}(D + L_c)\,(1 + I/K_i)}{u_{maxc} - (D + L_c)\,(1 + I/K_i)}$$

$$(11)\quad M_c = \frac{D\,(S_o - S)}{(D + L_c)}$$

$$(12)\quad M_c = \frac{D * S_o}{(D + L_c)} - \frac{D}{(D + L_c)} * \frac{K_{ms}\left[(D + L_c)\,(1 + I/K_i)\right]}{u_{maxc} - \left[(D + L_c)\,(1 + I/K_i)\right]}$$

$$(13)\quad M_c = \frac{D * S_o}{(D + L_c)} - \frac{D}{(D + L_c)} * (X) \text{ where X equals:}$$

$$\frac{K_{ms}\left[(D + L_c)\left\langle K_i\left[u_{maxh} - (D + L_h)\right] + K_{mi}(D + L_h)\right\rangle\right]}{u_{maxc} - \left[(D + L_c)\left\langle K_i\left[u_{maxh} - (D + L_h)\right] + K_{mi}(D + L_h)\right\rangle\right]}$$

zero. When equation (9) is solved for S, the result is equation (10). The steady state biomass is a function of the dilution rate, maintenance loss, initial substrate level, and the steady state substrate level and can be expressed by equation (11). Substituting equation (10) into equation (11) gives equation (12) which shows the relationship between the inhibitor level, the inhibitor constant, and the steady state biomass. However, (I) is again determined by the population of the heterotroph as given in equation (6). Therefore, equation (6) should be substituted for the value of (I) in equation (12). This results in equation (13) which could be simplified by combining terms and renaming them by definition. However, in its present form one can see that the steady state biomass of the chemolithotroph depends on the following physiological characteristics of the two microbes; the maximum growth rate of both the chemolithotroph and the heterotroph, the half saturation constants of both the chemolithotroph and the heterotroph, and the inhibitor constant of the chemolithotroph. In contrast, the steady state biomass of the heterotroph would depend only on the u_{max} and K_m for the heterotroph itself. Biomass of both the heterotroph and the chemolithotroph would, however, also depend on the yields and dilution rate.

SUMMARY AND CONCLUSIONS

A mathematical model was developed to express the potential relationship between the chemolithotroph, T. ferrooxidans, and a heterotroph, Acidiphilium sp., in the presence of a compound which inhibits T. ferrooxidans but is the growth substrate for the heterotroph. In the model, the growth of the chemolithotroph was strongly linked to the growth of the heterotroph. The model must now be verified by actual trials with the chemostat. If the model does not fit the data, the model must be altered. The key assumptions which may need to be changed are: (1) there is no substrate inhibition of the heterotroph, (2) the mechanism of inhibition of the chemolithotroph is noncompetitive, (3) there is no product inhibition, and (4) no other interactions exist between the two species.

Although the extent of inhibitory effects of organics on T. ferrooxidans in the natural environment is not known, Dispirito et al. (1981) have reported that a soluble component of pyrite was capable

of inhibiting the growth of T. ferrooxidans. Furthermore, it has already been established that obligate heterotrophs or mixotrophic thiobacilli capable of growing in an acidic environment can reduce the inhibitory effects of organics on T. ferrooxidans (Harrison 1984, Nerkar et al. 1977, Unz and Wichlacz 1982).

This model should also be generally applicable to situations where one organism metabolizes or transforms a substance which is inhibitory to a second organism. There are many such cases in the microbial world, including the ability of Beggiatoa to detoxify (oxidize) H_2S (Atlas and Bartha 1987) and the ability of chlorophenol-degrading bacteria to detoxify the environment for other microbes and for higher organisms (Steiert and Crawford 1985).

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